Rapid 2-hour workflow for detection of select bacteria in stool samples

C. difficile detection

The human digestive tract is home to over 1,000 different species of microbes, including bacteria, archaea, fungi, protists, and viruses [1,2]. Most of them are harmless, or actually beneficial, to human health. However, when the balance of organisms in the gut is disrupted by antibiotic use, certain bacteria can grow out of control and make their host sick. The bacterium *Clostridium difficile* is a common cause of illness associated with the use of antibiotics [3]. As the bacteria overgrow and replace normal gut flora, they produce toxins damaging the lining of the large intestine, causing severe diarrhea, and sometimes fever, abdominal pain, nausea, and vomiting that can persist for many days.

C. difficile causes >450,000 infections in the United States every year, according to the Centers for Disease Control and Prevention [4]. People aged 65 years or older, who take antibiotics, are at the highest risk for developing *C. difficile* infections. Approximately 29,000 people die within 30 days of the initial diagnosis; of those, 15,000 deaths are estimated to be directly attributable to *C. difficile* infections.

Purification of microbial DNA from a sample is a key step in understanding the microbiome composition and its influences on human health. Following DNA purification, PCR-based molecular assays offer a very sensitive, accurate, and rapid approach to detect *C. difficile* and other bacteria for a variety of research applications. Here we describe a workflow for robust isolation of aggregate microbial and host DNA from stool samples, followed by qPCR utilizing Applied Biosystems™ TaqMan™ Assays. This workflow requires as little as 2 hours to complete (Figure 1).

Sample

PureLink Microbiome DNA Purification Kit

QuantStudio system or other qPCR instrument

Figure 1. Workflow for rapid detection of medically relevant bacteria in stool samples for research. This workflow can be performed in as little as 2 hours with an Applied Biosystems™ QuantStudio™ real-time PCR system or other qPCR instrument in Fast mode.

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The key component of the workflow is the Invitrogen™ PureLink™ Microbiome DNA Purification Kit. This kit enables fast purification of high-quality microbial and host DNA from several sample types, including particularly challenging stool samples. The kit uses proven Invitrogen™ PureLink™ spin column technology for excellent yield of purified DNA that is ready for downstream PCR, sequencing, or other applications. Highly efficient cell lysis, fast removal of inhibitors, and versatility of sample types make this a superior kit for rapid detection of medically relevant bacteria.

Features of the PureLink Microbiome DNA Purification Kit include:

- Efficient lysis of all microorganisms (including durable species with thicker and more complex cell walls) by a combination of heat, chemical disruption, and mechanical disruption with specialized beads
- Minimization of inhibitory compounds by precipitation using a proprietary clean-up buffer
- Streamlined protocols for stool as well as urine, saliva, and swabs
- Recovery of highly pure DNA compatible with common downstream applications such as qPCR and next-generation sequencing

Once the stool-derived DNA is purified, the next step is qPCR detection of specific bacteria or other microorganisms with corresponding TaqMan Assays. This step can take as little as 45 minutes with QuantStudio real-time PCR systems in Fast mode.

Following the workflow described above, total DNA was isolated from the stool samples of 5 donors with *C. difficile* infection, and 4 healthy donors as controls. Next, *C. difficile* was detected by qPCR using a corresponding TaqMan Assay. Figure 2 shows the results of this experiment: for all healthy donor samples and negative control sample (no DNA input), C_t values were >40 (undetermined), while for all samples derived from donors with *C. difficile* infection, C_t values were in the 23–27 range, indicating very efficient detection of this bacterium. This clearly demonstrates the utility of the workflow for robust detection of *C. difficile* in stool-derived samples, and efficient discrimination from control samples derived from healthy donors.

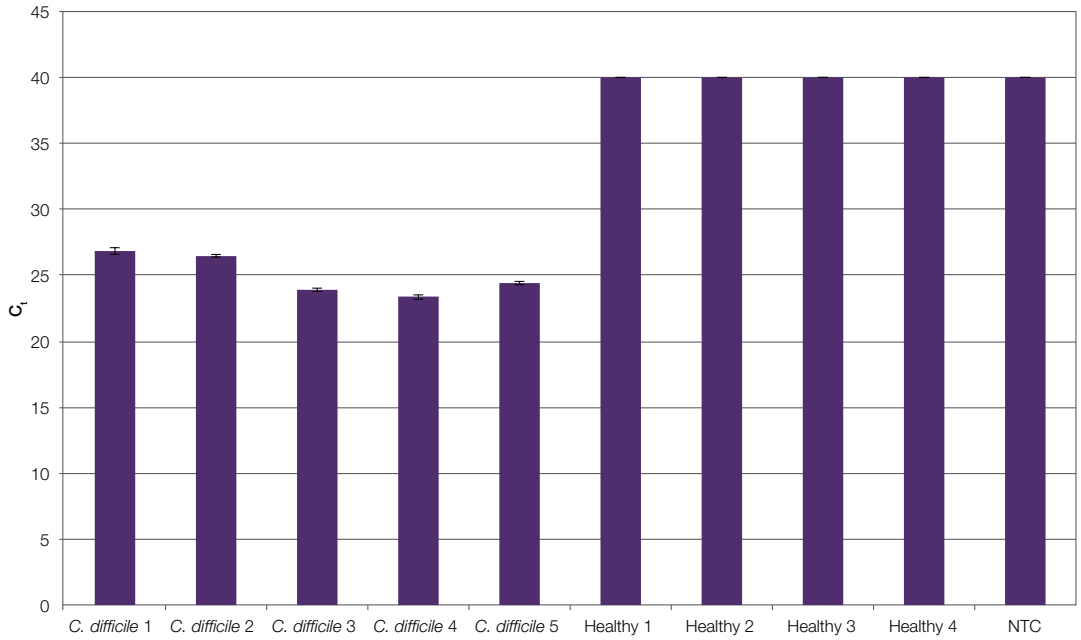

Figure 2. Robust detection of *C. difficile* in human stool samples. DNA was isolated using the PureLink Microbiome DNA Purification Kit from 0.2 g stool samples that were obtained from 5 donors with *C. difficile* infection and 4 healthy donors as controls. qPCR with a TaqMan Assay specific to *C. difficile* was then performed. NTC: no-template control (no DNA input).

Salmonella detection

Another example of a medically relevant bacterium is *Salmonella*. Every year, *Salmonella* is estimated to cause 1.2 million foodborne illnesses in the United States alone, with 23,000 hospitalizations and 450 deaths [5]. Most people infected with *Salmonella* develop diarrhea, fever, and abdominal cramps 12–72 hours after infection. Even though the vast majority of affected individuals recover within a week, in some cases hospitalization and urgent treatment is required.

As with *C. difficile*, PCR-based molecular assays enable *Salmonella* detection with high sensitivity, specificity, and speed—capable of providing results in a single day. Following the workflow described above, total DNA was isolated from the stool samples of 2 donors with *Salmonella* infection, and 4 healthy donors as controls. Next, *Salmonella* was detected by qPCR using a corresponding TaqMan Assay. Figure 3 shows the results of this experiment: for all healthy donor samples and negative control sample (no DNA input), C_t values were >40 (undetermined), while for both samples derived from donors with *Salmonella* infection, C_t values were in the 16–18 range, indicating very efficient detection of this bacterium. This clearly demonstrates the utility of the workflow for robust detection of *Salmonella* in stool-derived samples.

Conclusions

We describe a workflow for rapid detection of medically relevant bacteria in human stool samples, starting with robust isolation of high-quality, inhibitor-free DNA with the PureLink Microbiome DNA Purification Kit, to downstream analysis by qPCR. This workflow provides a superior alternative to current assays (like bacterial culture) for detection of *C. difficile*, *Salmonella*, and other microorganisms in stool, and can also be utilized with other clinical research samples.

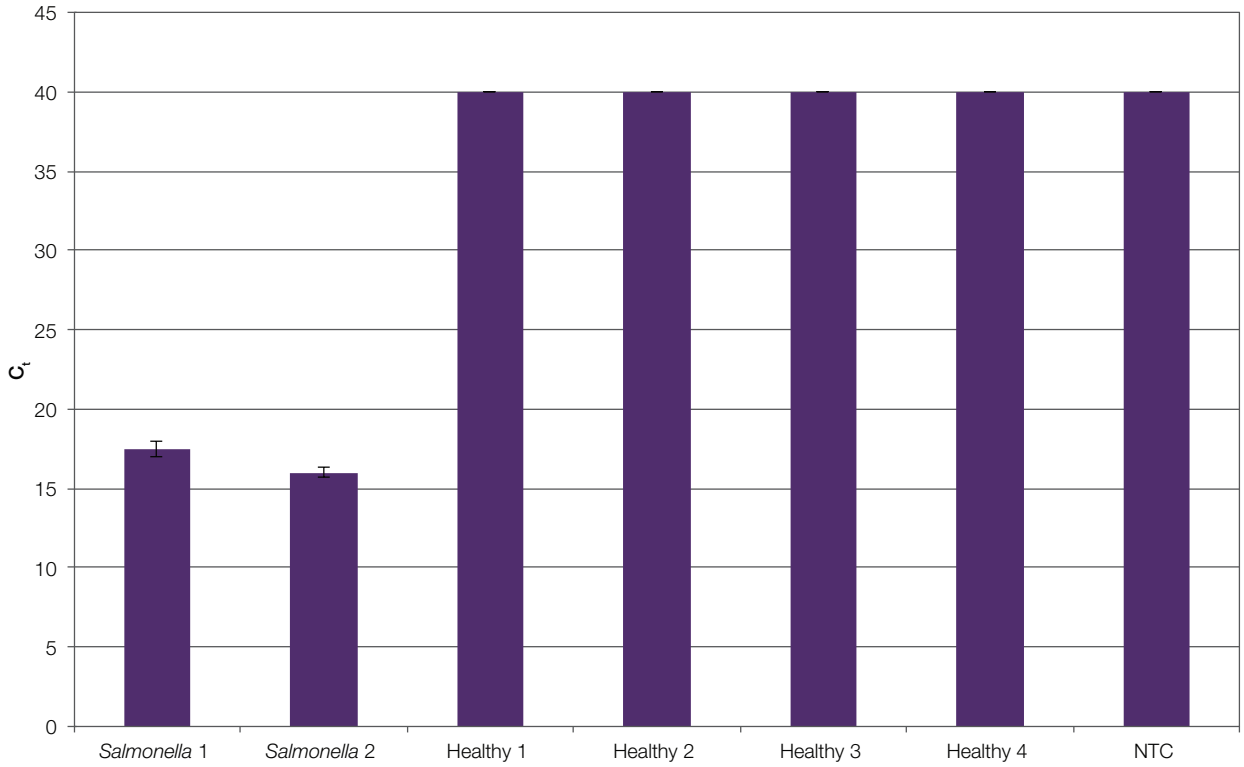

Figure 3. Robust detection of *Salmonella* in human stool samples. DNA was isolated using the PureLink Microbiome DNA Purification Kit from 0.2 g stool samples that were obtained from 2 donors with *Salmonella* infection and 4 healthy donors as controls. qPCR with a TaqMan Assay specific to *Salmonella* was then performed. NTC: no-template control (no DNA input).

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References

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